PATENT Case 5400/2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF	
S. BERTENSHAW ET AL	GROUP ART UNIT: 120
SERIAL NO.: 08/425,022	EXAMINER: DENTZ
FILED: April 19, 1995	DATE: April 14, 1997

TITLE: SUBSTITUTED FURANS AND FURANONES FOR THE TREATMENT OF INFLAMMATION

DECLARATION UNDER 37 C.F.R. §1.132

The Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

I, John Likos, Ph.D., declare that:

1. I received a Bachelor of Science Degree in Biology from Loyola University of Los Angeles in 1968, a Ph.D. in Biochemistry from Iowa State University in 1976, and from 1977 to 1980 I was an NIH post-doctoral fellow at the University of Delaware;

2. Since 1980, I have been employed at Monsanto Company, Saint Louis, Missouri, and currently I hold the position of Senior Research Specialist, with responsibility for the application of nuclear magnetic resonance (NMR) techniques to chemical and biological systems;

3. I am the principal author or co-author of approximately 10 publications;

4. In my professional capacities, I closely and carefully follow the scientific literature regarding organic chemistry, bio-organic chemistry and specifically NMR methods;

5. Exhibit A, attached hereto, includes copies of selected NMR reports from studies I made on the base-catalyzed keto-enol tautomerism of substituted 4-(4-methylsulfonyl)phenyl-2H-furanones. All studies were performed on a Varian UNITY-INOVA 600 MHz spectrometer equipped with a 5mm triple-resonance gradient, variable temperature probe. The studies were performed in full accordance with good laboratory practices. The samples were maintained at 30±1 °C. Spectra were referenced to the solvent (2.47 ppm for d_6-DMSO and 3.30 ppm for d_4-MeOH). In general, the substituted 2H-furanone samples (~6 mg) were dissolved in d_6-DMSO (0.85 ml) and diluted with water (0.1 ml).

I have found the following results:

a. On pages A1-A5, NMR data are shown pertaining to the spectral assignment and characterization of the tautomers of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone:

i. On page A1, the NMR spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone (6 mg) in d_6-DMSO/H_2O (89.5/10.5 v/v%) (A1(a)) is shown. Proton resonances are observed at δ =7.87 (d, 2H, J = 8.53 Hz.) and 7.56 (d, 2H, J = 8.53 Hz.) (assigned to the 4-(4-methylsulfonyl)phenyl group), 7.38 (m, 3H) and 7.28 (m, 2H) (assigned to the 3-phenyl group), 5.34 (s, 2H) (assigned to H5), and 3.16 (s, 3H) (assigned to the methylsulfonyl). The chemical shifts, J-couplings and integration of these resonances are consistent with the keto tautomer. Upon addition of base, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (~1.4 equiv.), two distinct sets of resonances are observed (A1(b)). In addition to the keto tautomer resonances described above, proton resonances are observed at δ =7.71 (d, 2H, J = 8.38 Hz,) and 7.34 (d, 2H, J = 8.38 Hz.) (assigned to the 4-(4-methylsulfonyl)phenyl group), 7.12 (d, 2H, J = 7.52 Hz.) (assigned to H2 and H6 of the 3-phenyl group), 6.96 (t, 2H, J = 7.52 Hz.)

(assigned to H3 and H5 of the 3-phenyl group), 6.66 (t, 1H, J = 7.52 Hz.) (assigned to H4 of the 3-phenyl group), 6.55 (s, 1H) (assigned to H5 of the furanol ring) and 3.12 (s, 3H) (assigned to the methylsulfonyl). The chemical shifts, J-couplings and integration of these resonances are consistent with the enol tautomer. Approximately 30% enol is detected relative to the keto form under these conditions;

ii. On page A2, a ^{1}H-COSY (correlated spectroscopy) spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone (6 mg) and DBU (~1.4 equiv.) in d_6-DMSO/$H_2$0 (89.5/10.5 v/v%) is shown. The proton coupling patterns for the aromatic rings are indicated for the furanone (solid line) and the furanol (dashed line). The coupling patterns indicate that both keto and enol forms are present, consistent with the assignments made in paragraph 5(a)(i);

iii. On page A3, the DEPT-HMQC spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone (6 mg) and DBU (~1.4 equiv.) in d_6-DMSO/$H_2$0 (89.5/10.5 v/v%) indicates that the resonance at 6.55 ppm is a methine (CH) proton (A3(a)). This study confirms the assignment of the furanol H5 proton;

iv. On page A4, a NOESY spectrum (NOE spectroscopy) of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O is shown. This study measures the transfer of longitudinal magnetization that can occur by either cross-relaxation (protons close together in space (<40-50 nm) produce negative cross peaks) or chemical exchange (protons produce positive cross peaks). All NOE cross-peaks shown in A4 are negative, indicating protons close together in space. The cross-peaks between the CH_3 group (3.16ppm) and the resonance at

7.87 ppm indicates that this resonance is H5 and H3 of the 4-(4-methylsulfonyl)phenyl group due to their proximity to the methylsulfonyl protons. Likewise, the resonance at 7.56 ppm is assigned to H2 and H6 of the 4-(4-methylsulfonyl)phenyl group due to the proximity to the furanone H5 protons. In addition, the cross-peak between H2 and H6 of the 4-(4-methylsulfonyl)phenyl (7.56 ppm) to the resonance at 7.28 ppm suggests this resonance is H2 and H6 of the 3-phenyl group. These assignments are consistent with and extend the assignments presented above for the keto tautomer form;

v. On page A5, a NOESY spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O and DBU (~0.7 equiv.) is shown. Approximately 20% enol is observed. In addition to the negative NOE peaks observed for the keto form, positive cross-peaks are observed, indicating chemical exchange between structurally-related protons of the enol/keto tautomers. This study indicates the enol/keto tautomers are interconverting;

b. On pages A6-A7, NMR data are shown pertaining to the formation and spectral characteristics of the furanol and unconjugated keto forms of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone:

i. On page A6, the NMR spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%) and NaOH (~0.5 equiv.) is shown. Two distinct sets of resonances are observed that correlate with both furanone and furanol forms. Approximately 20% enol is detected relative to the keto form under these conditions. Upon neutralizing this solution with HCl (~0.5 equiv.), the enol resonances decrease and two distinct sets of peaks are observed

(6(b)) that correlate with two furanone forms. In addition to the previously described conjugated ($\alpha\beta$) keto form, the unconjugated ($\beta\gamma$) keto form is observed with proton resonances at δ =8.08 (d, 1H, J = 2.1Hz.) and 5.27 (d, 1H, J = 2.1 Hz.) (assigned to the H3/H5 pair of protons), 7.74 (d, 2H, J = 8.76 Hz.) and 7.52 (d, 2H, J = 8.76 Hz.) (assigned to the 4-(4-methylsulfonyl)phenyl group), 7.32 (m) and 7.25 (m) (assigned to the 3-phenyl group), and 3.08 (s, 3H) (assigned to the methylsulfonyl group). The chemical shifts, J-couplings and integration of these new resonances are consistent with the unconjugated keto form;

ii. On page A7, the DEPT-HMQC spectrum (A7(a)) of the neutralized mixture indicates that the resonances at 8.08 and 5.27 ppm are both methine protons (CH), consistent with their assignment in the unconjugated keto form;

c. On pages A8-A9, NMR data are given pertaining to spectral characterization of the tautomers of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone:

i. On page A8, the NMR spectrum of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%) is shown (A8(a)). The keto tautomer is observed with resonances at δ =8.23 (d, 2H, J = 8.89 Hz.), 7.57 (d, 2H, J = 8.89 Hz.), 7.89 (d, 2H, J = 8.53 Hz.), and 7.56 (d, 2H, J = 8.53 Hz.) (assigned to the aryl protons), 5.39 (s, 2H) (assigned to H5), and 3.16 (s, 3H) (assigned to the methylsulfonyl group). Upon addition of DBU (~0.8 equiv.), a second distinct set of signals are observed at δ =7.81 (d, 2H, J = 8.37 Hz.), 7.41 (d, 2H, J = 8.37 Hz.), 7.72 (d, 2H, J = 9.20 Hz.), and 7.26 (d, 2H, J = 9.20 Hz.) (assigned to the aryl

protons), 6.68 (s, 1H) (assigned to H5 of the furanol ring), and 3.15 (s, 3H) (assigned to the methylsulfonyl group). The chemical shifts, J-couplings and integration are consistent with the enol tautomer. Approximately 80% enol is observed relative to the keto form under these conditions;

ii. On page A9, the DEPT-HMQC spectrum (A9(a)) of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone and DBU (~0.8 equiv.) in d_6-DMSO/H_2O (89.5/10.5 v/v%) indicates that the resonance at 6.68 ppm is a methine (CH) proton consistent with its assignment as H5 of the enol tautomer;

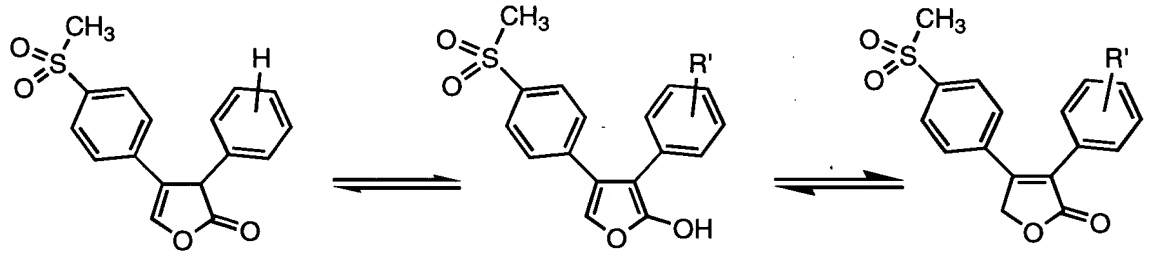
d. On page A10, the NMR data is given pertaining to the spectral characterization of the tautomers of 3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone. In A10(a), the NMR spectrum of 3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10/5 v/v%) is shown. Signals attributed to the keto tautomer are observed at δ =7.88 (d, 2H, J = 8.70 Hz.) and 7.55 (d, 2H, J = 8.70Hz.) (assigned to the 4-(4-methylsulfonyl)phenyl group), 7.33 (q, 2H, J_{HH} = 8.84 Hz., J_{HF} = 5.52 Hz.) and 7.21 (t, 2H, J_{HH} = 8.84 Hz., J_{HF} = 8.84 Hz.) (assigned to the 3-(4-fluorophenyl) group), 5.32 (s, 2H) (assigned to H5), and 3.16 (s, 3H) (assigned to the methylsulfonyl group). Upon addition of DBU (~0.7 equiv.), a second distinct set of signals is observed at δ=7.71 (d, 2H, J = 8.41 Hz.) and 7.32 (d, J = 8.41 Hz.) (assigned to the 4-(4-methylsulfonyl)phenyl group), 7.09 (q, 2H, J_{HH} = 9.04 Hz., J_{HF} = 5.74 Hz.) and 6.78 (t, 2H, J_{HH} = 9.04 Hz., J_{HF} = 9.04 Hz.) (assigned to the 3-(4-fluorophenyl)group), 6.54 (s, 1H) (assigned to H5), and 3.11 (s, 3H) (assigned to the methylsulfonyl group). The chemical shifts, J-couplings and integration are consistent with the enol tautomer. Under these conditions, ~30% enol is observed relative to the keto tautomer form;

e. On page A11, NMR data pertaining to the deuterium exchange reaction of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone are given. The deuterium exchange was followed at pH 7.5 on a Varian UNITY-INOVA 600 MHz spectrometer. A stock solution of 100 mM sodium phosphate was prepared from D_3PO_4 in D_2O and titrated with NaOD to pH 7.5 (uncorrected). The furanone (1 mg) was dissolved in d_4-MeOH/D_2O (67/33 v/v%)(0.8 ml) and the above buffer was added (0.4 ml). For the control, D_20 (0.4 ml) was used as the aqueous component. On page A11(a) the NMR signal changes observed during the course of the reaction are shown. The signal intensity of the H5 protons (5.40 ppm) of the keto tautomer decreases with time while a signal at 5.38 ppm initially increases then decreases. This resonance is assigned to the H5 mono-deuterated keto tautomer. The signal intensities are normalized to that of the H3 and H5 resonance of the 4-(4-methylsulfonyl)phenyl group which does not change in intensity with time. A plot of these normalized intensity changes is shown in A11(b). These data are consistent with the formation of the enol tautomer during the exchange reaction;

f. On page A12, NMR data pertaining to the deuterium exchange reaction of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone are given. The deuterium exchange was followed at pH 7.5 on a Varian UNITY-INOVA 600 MHz spectrometer. The compound (1 mg) was dissolved in d_6-DMSO (0.6 ml) and diluted with deuterated phosphate buffer (pH 7.5) (0.15 ml), described in paragraph 5.e, above. For the control, d_6-DMSO (0.6 ml) was diluted with 100 mM phosphate buffer (pH 7.5) (0.15 ml). The control phosphate buffer was prepared by dissolving H_3PO_4 in H_2O and adjusting the pH to 7.5 with sodium hydroxide. Figure A12(a) shows the spectral changes observed during the course of the reaction. The signal intensity of the H5 protons (5.40 ppm) of both the keto and furanol tautomers decrease with time, while the signal assigned to the H5 mono-deuterated carbonyl tautomer (5.38 ppm) initially increases then decreases. The keto 5-CH2 and 5-CHD intensities

were normalized to that of the H3,H5 resonance of the 4-(4-methylsulfonyl)phenyl group of the keto tautomer; the enol 5-CH intensity was normalized to the same resonance of the enol tautomer. A plot of these intensity changes is shown in A12(b). These data shows the compound incorporates deuterium at position C5 of the furanone, which is consistent with the formation of a 2-hydroxy compound during the exchange reaction;

6. Based on my analysis, we have observed the three tautomeric structures shown below (where R' is fluoro, hydrogen or nitro):



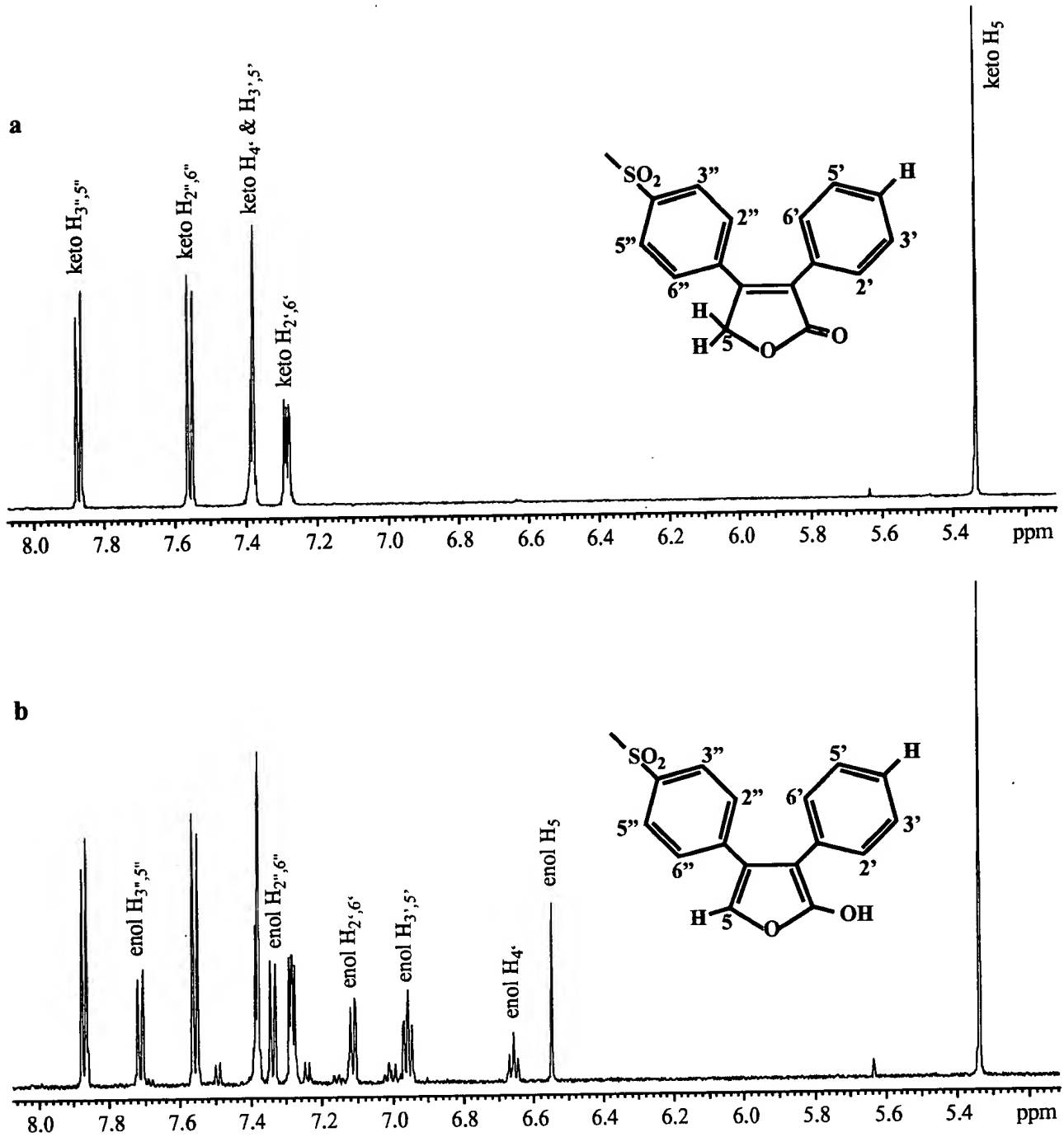
keto enol keto

I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

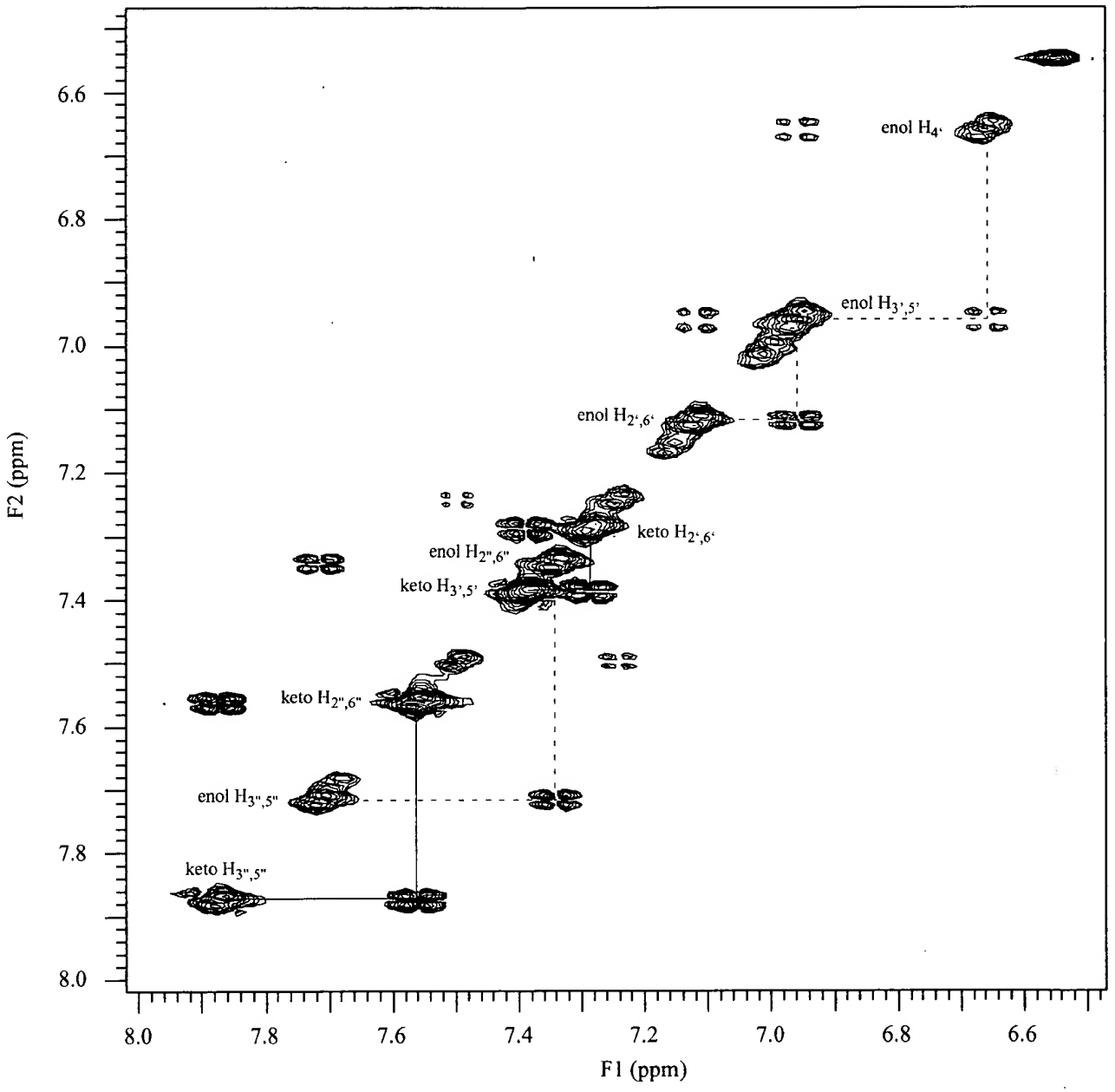
Respectfully submitted

4/14/97
Date

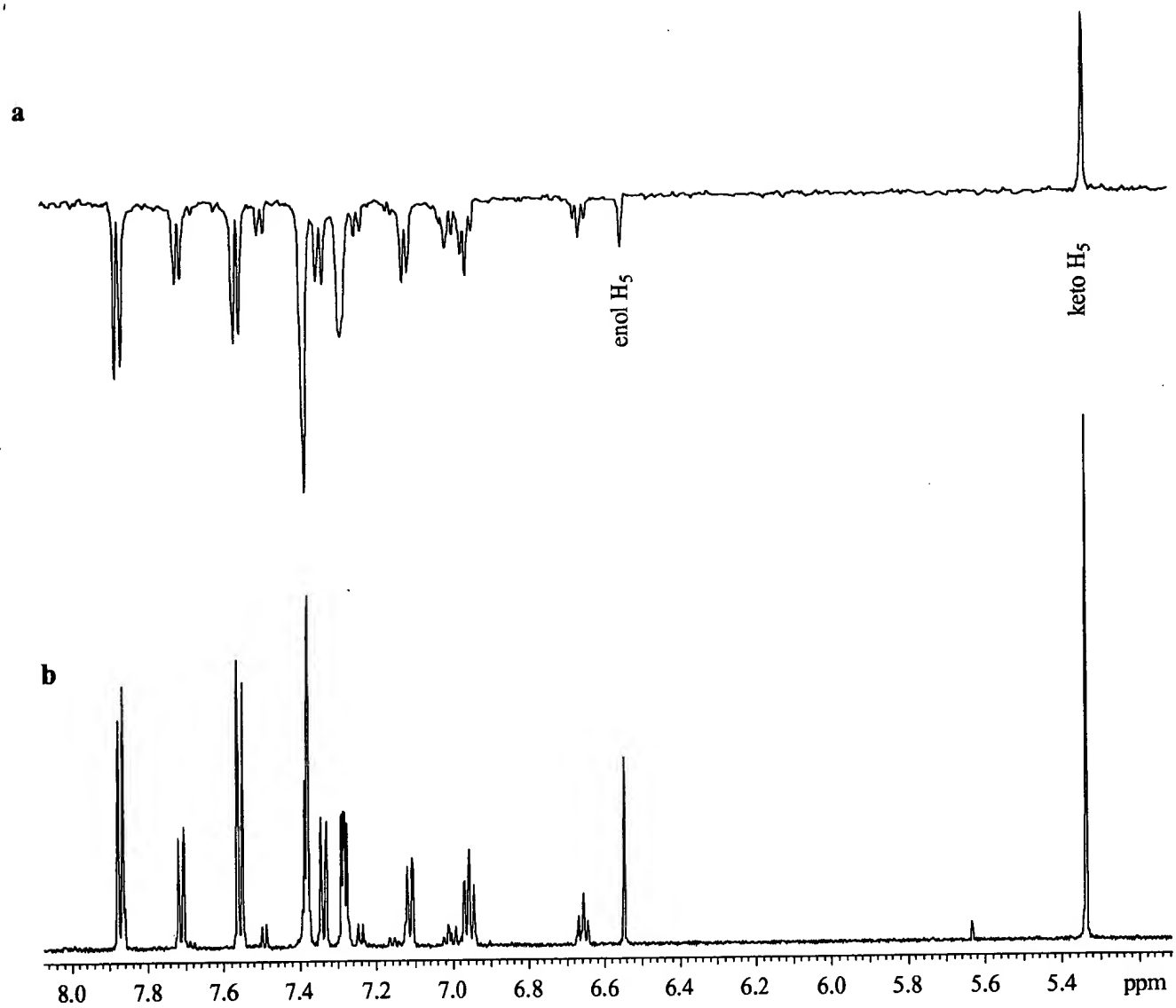
John Likos, Ph.D.



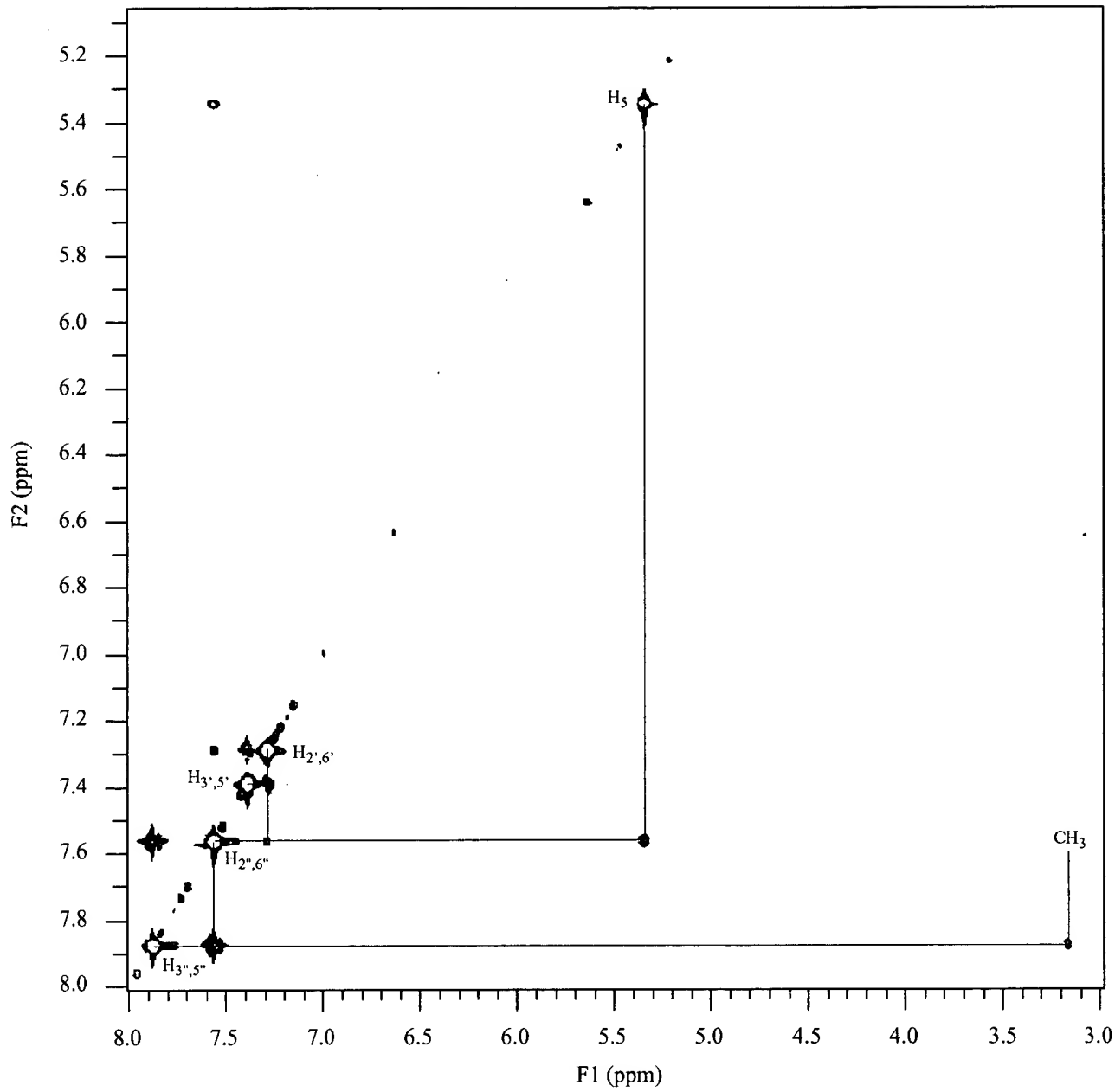
a. The ^{1}H NMR spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%);
b. after addition of DBU (~1.4equiv.) to (a).



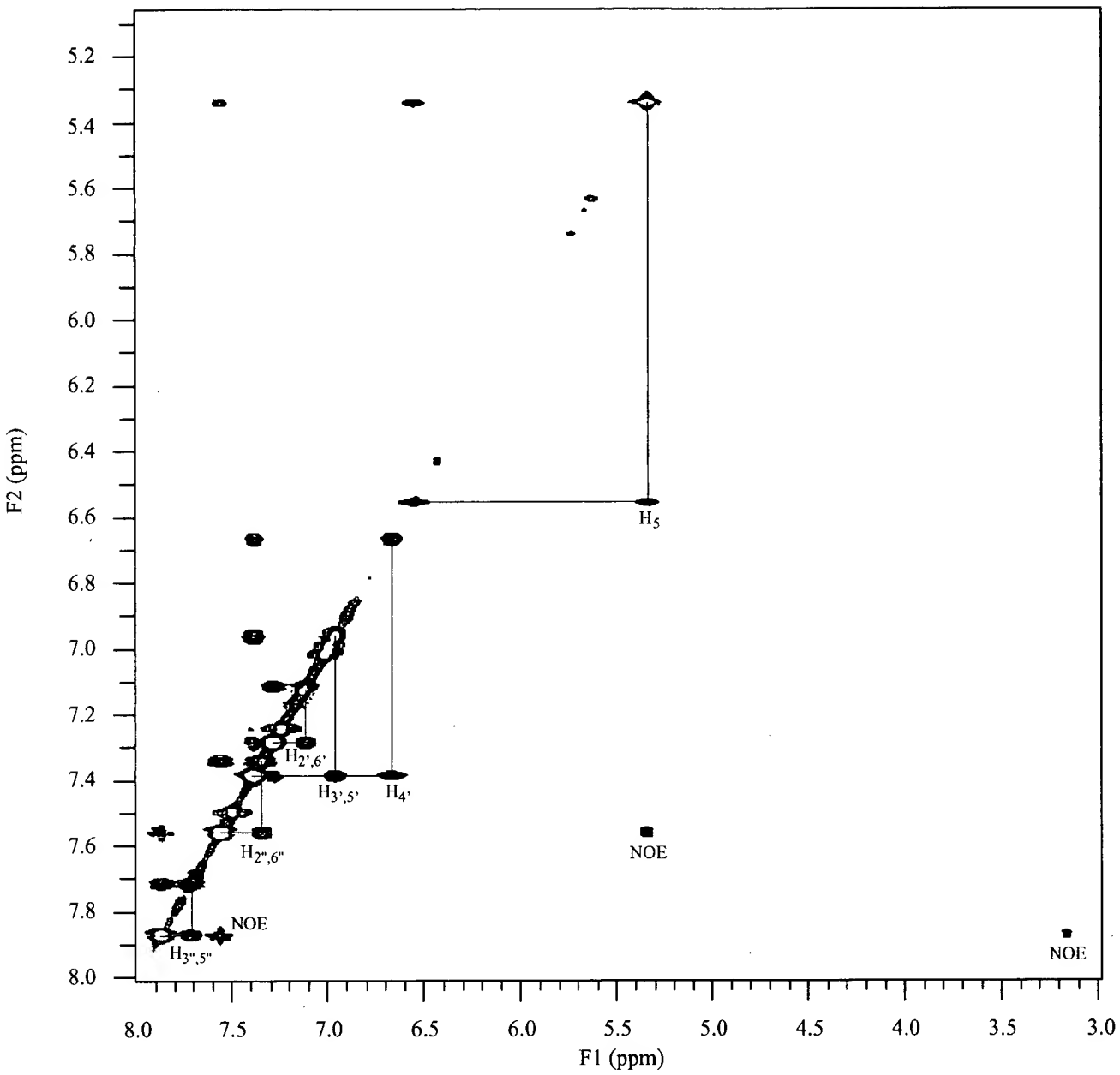
The ^{1}H-COSY spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%) after addition of DBU (~1.4equiv.). The solid lines indicate the aromatic ^{1}H couplings in the keto form; the dashed lines, in the enol.



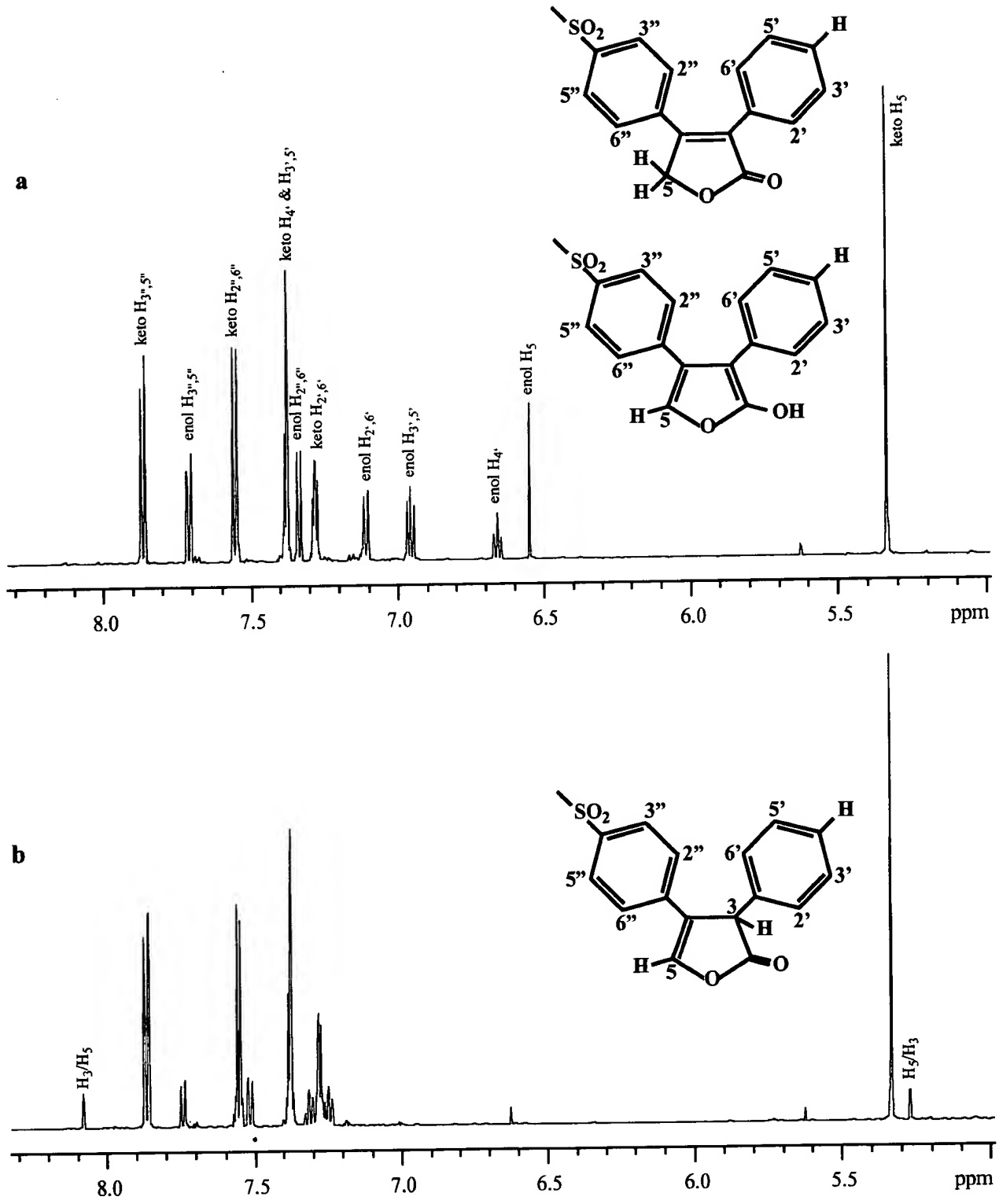
a. The ^{1}H-DEPT-HMQC spectrum of mixture from A1(b); CH_2 groups are up, CH and CH_3 down;
b. spectrum of A1(b) shown for reference.



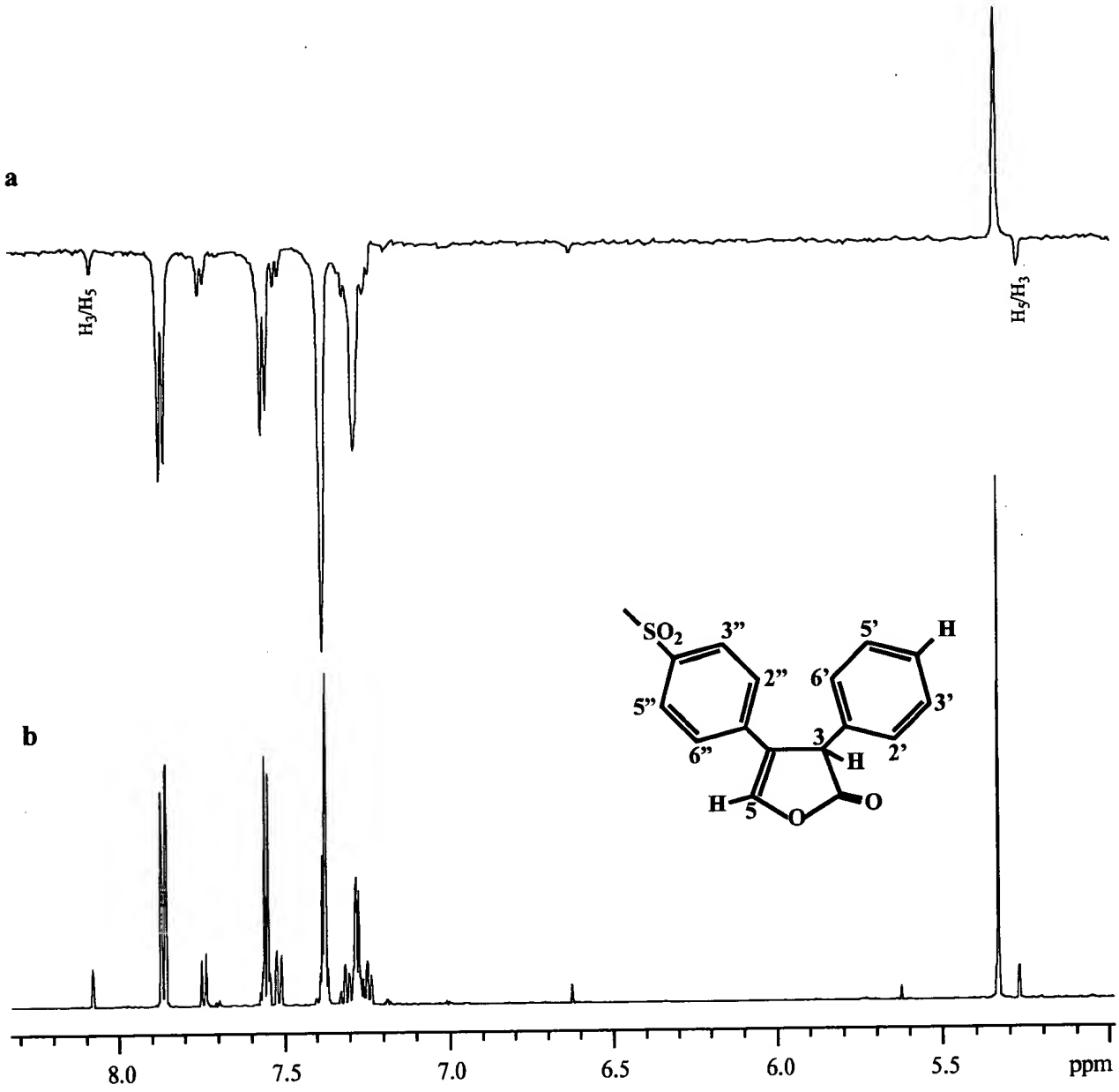
The ^{1}H-NOESY spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%).



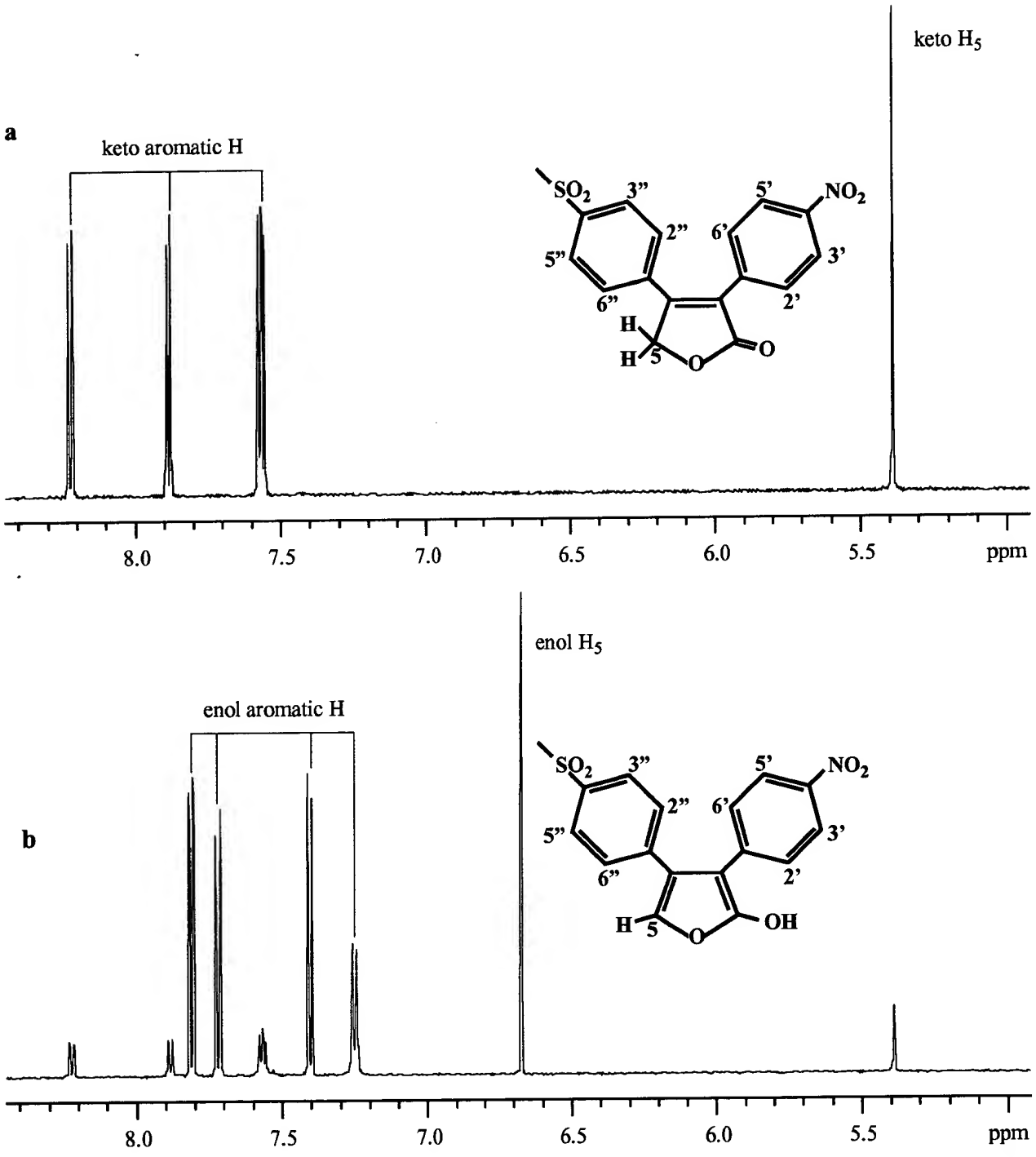
The ^{1}H-NOESY spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%) after addition of DBU (~0.7 equiv.). The lines connect pairs of keto/enol protons whose longitudinal magnetization is mixed by two-site chemical exchange.



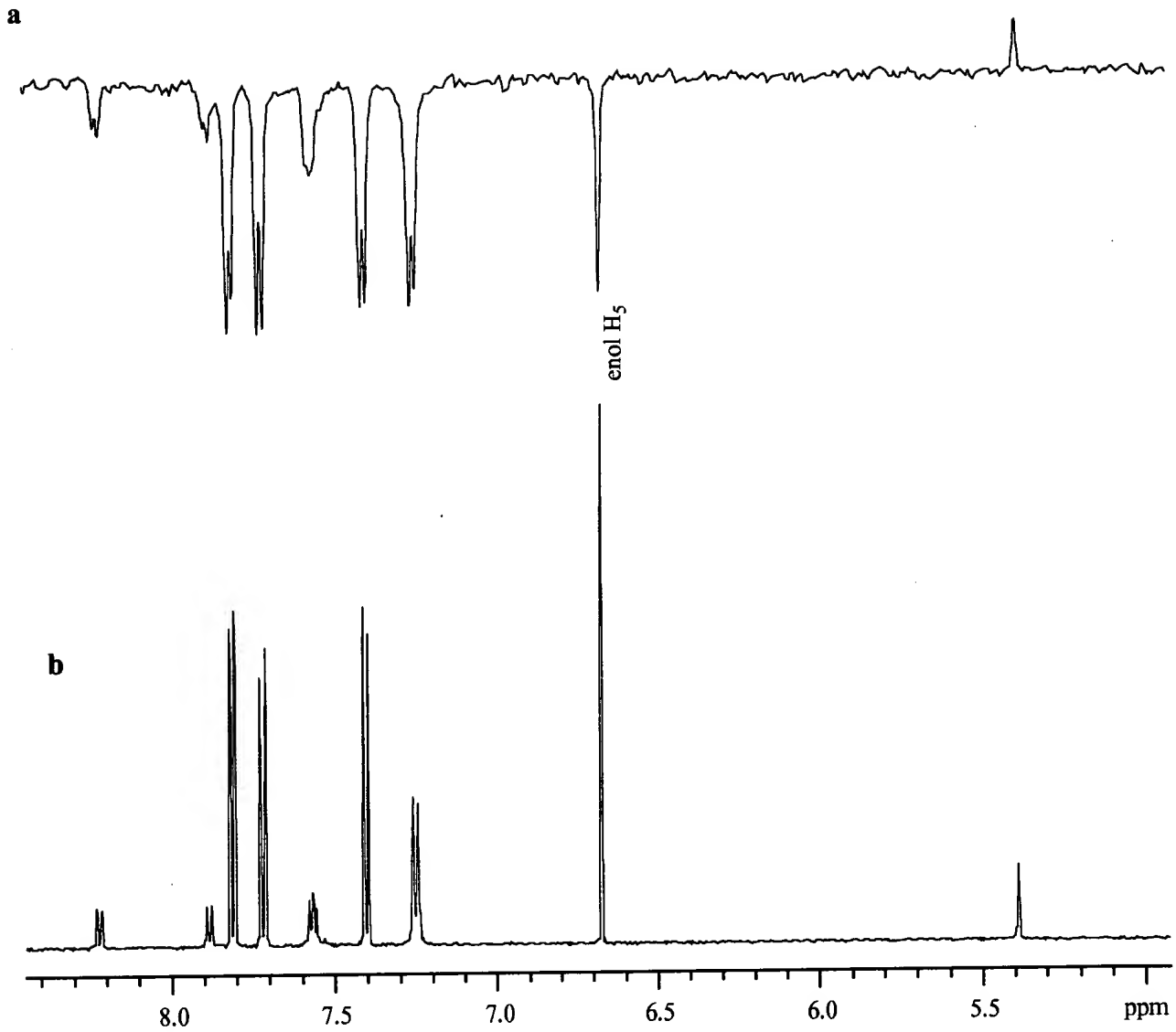
a. The ^{1}H-NMR spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%) after addition of NaOH (~0.5 equiv.);
b. after neutralizing (a) with HCl.



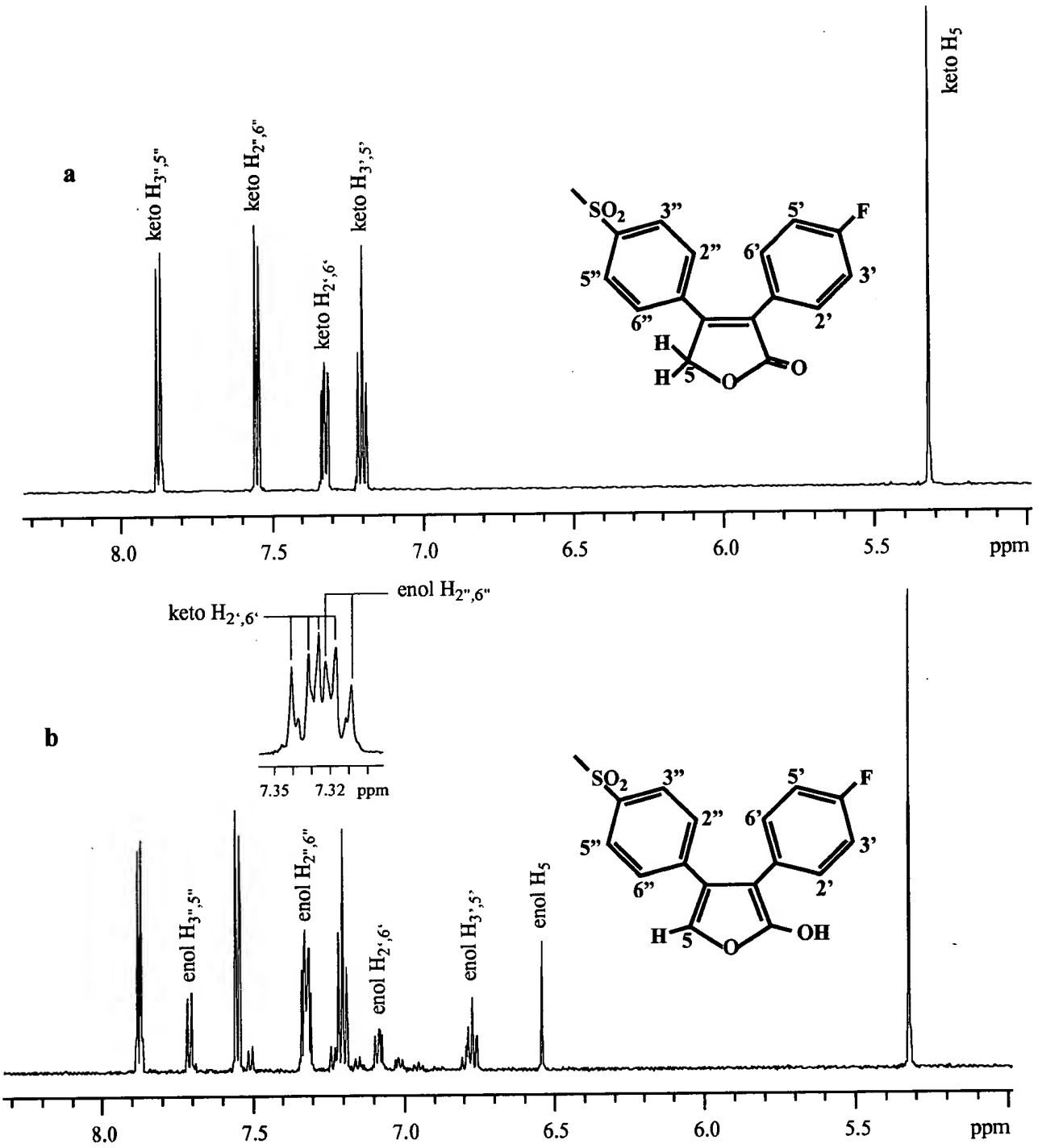
a. The ^{1}H-DEPT-HMQC spectrum of the mixture from A6(b); CH_2 groups are up, CH and CH_3 down;
b. spectrum of A6(b) shown for reference.



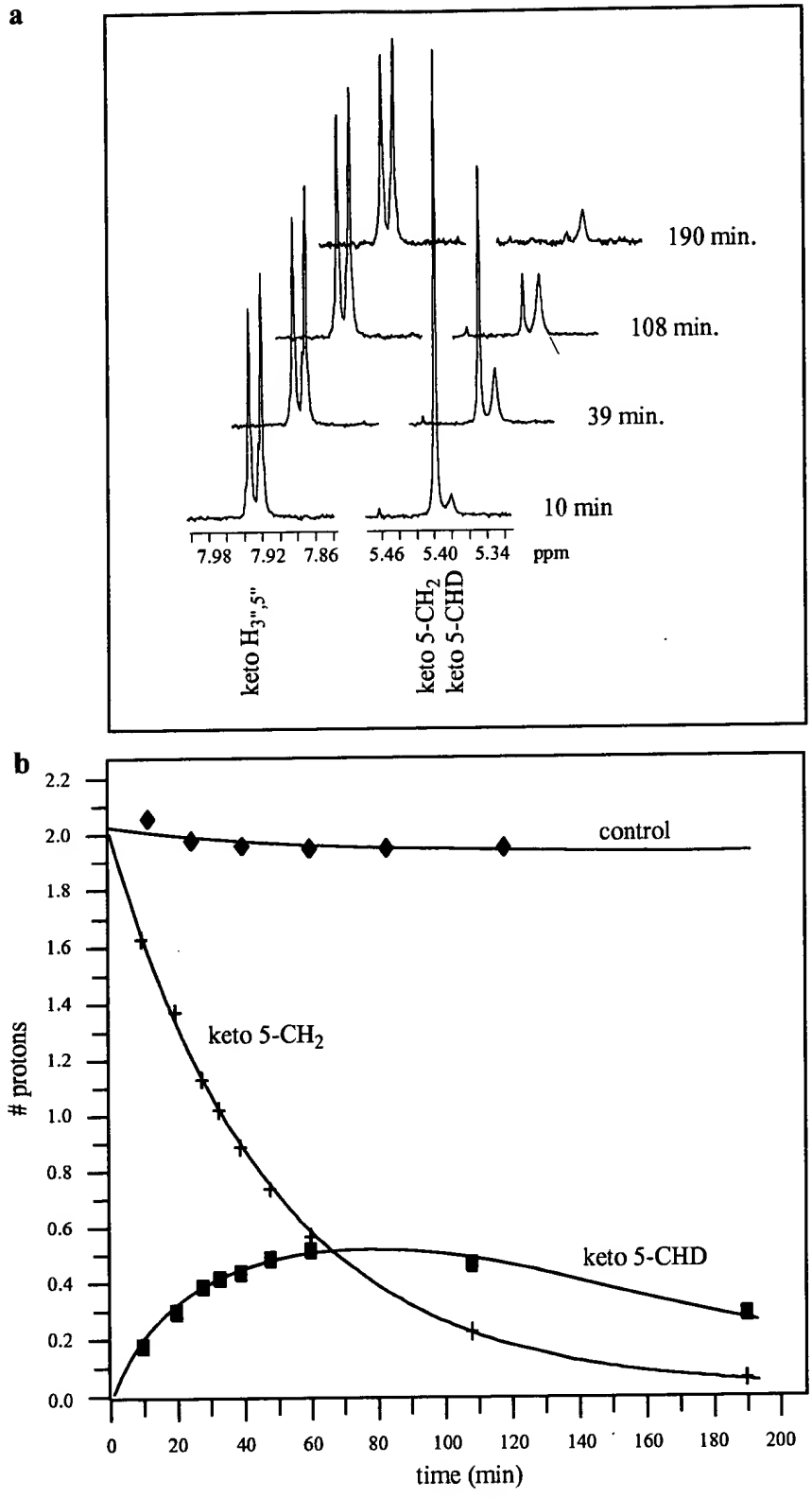
a. The ^1H NMR spectrum of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%);
b. after addition of DBU (~ 0.8 equiv.) to (a).



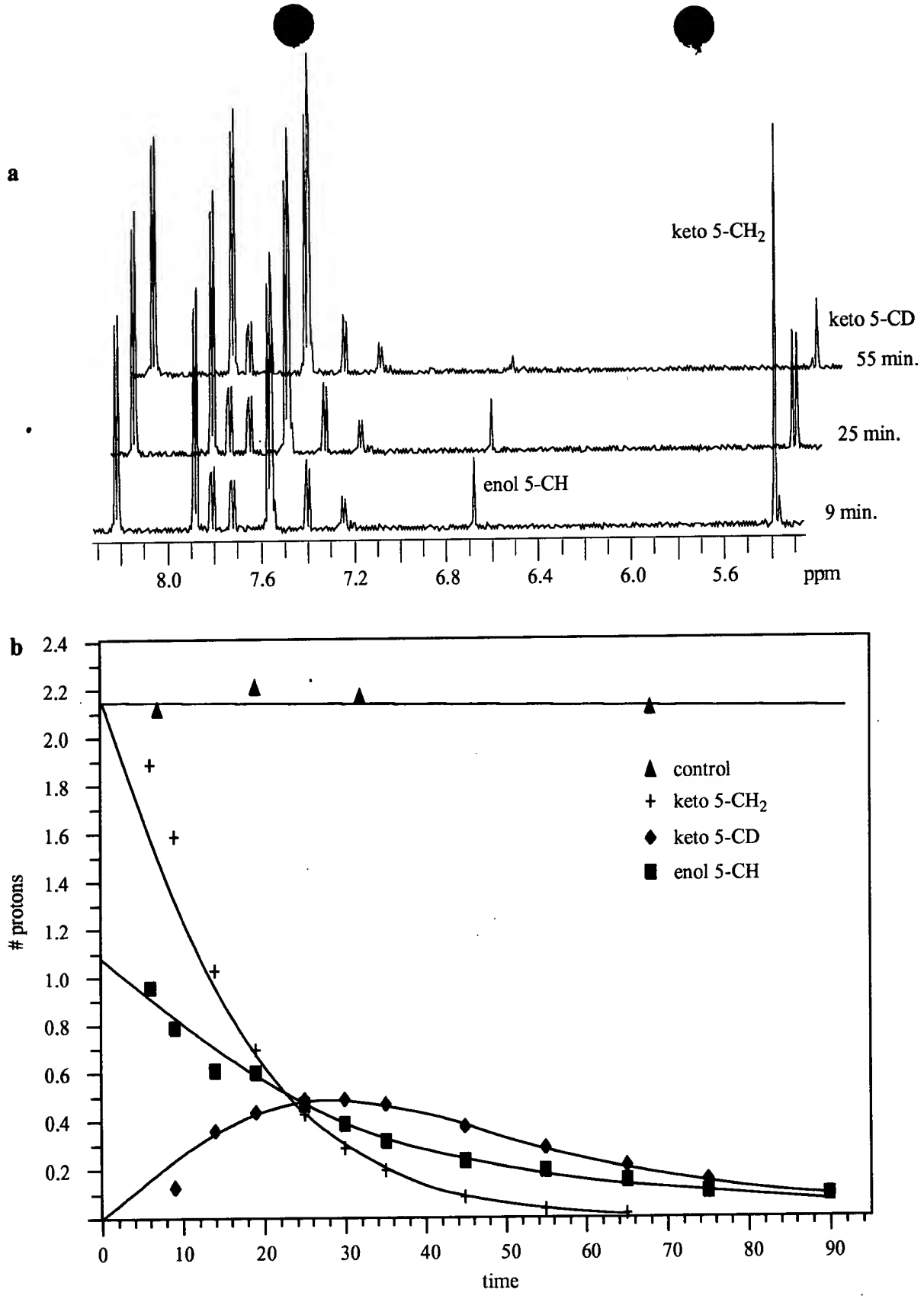
a. The ^{1}H-DEPT-HMQC spectrum of the mixture from A8(b); CH_2 groups are up, CH and CH_3 down; **b.** spectrum of A8(b) shown for reference.



a. The ^{1}H NMR spectrum of 3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%);
b. after the addition of DBU (~0.7 equiv.) to (a). The inset is an expansion of the multiplet at 7.32 ppm.



Deuterium exchange of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_4-MeOH/D_2O (67/33 v/v%) and 33 mM sodium phosphate, pH 7.5; (**a**) observed changes in signal intensity for the 5-CH_2 (5.40 ppm) and 5-CHD (5.38 ppm) protons of the keto form; (**b**) time course of (a).



Deuterium exchange of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone in d_6-DMSO/D_2O (80/20 v/v%) and 20 mM sodium phosphate, pH 7.5; (**a**) observed changes in signal intensity for the keto 5-CH_2 (5.40 ppm) , keto 5-CHD (5.38 ppm) and enol 5-CH (6.68 ppm) protons; (**b**) time course of (a).